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**Review: Comprehensive analysis of molecular phylogeographic structure among the meadow jumping mice (*Zapus hudsonius*) reveals evolutionarily distinct subspecies (T. L. King, J. F. Switzer, C. L. Morrison, M. S. Eackles, C. C. Young, B. Lubinski, and P. Cryan)**

King et al. have successfully addressed all of the criticisms directed at the study design, scope of analysis, and analytical techniques that were directed at Ramey et al. (2005). In doing so, they have produced strong and conclusive results that fully contradict those of Ramey et al. (2005). King et al. demonstrate not only that *Zapus hudsonius preblei* is a distinct subspecies, but also that there are two genetic units within *Z. h. preblei* that probably warrant separate conservation status. I am very impressed with the comprehensive nature of this study, and fully support the conclusions and interpretations.

In reviewing the history of the Ramey et al. study, I note that most of the reviewers of the initial report (including me) agreed in general with the techniques employed and taxonomic conclusions. At that time, most of the disagreement dealt with the conservation interpretations of those taxonomic conclusions, as well as the condescending tone of the paper. Indeed, it was probably the unnecessarily insulting tone of the paper as much as the conservation implications that encouraged closer scrutiny of the genetic techniques employed by Ramey et al. Whatever the motivation, closer inspection revealed some very basic flaws in the study design, scope of analysis, and analytical techniques of Ramey et al. Most important to me was that Ramey et al. appeared to be analyzing these subspecies using criteria that applied to species: they expected no introgressive hybridization and complete sorting of mtDNA haplotypes among subspecies, several of which co-occurred along broad fronts. Many of the samples used by Ramey et al. were from subspecies boundaries, and so were obviously subject to misidentification, introgression, and migration. Moreover, the expectation of complete sorting was absurd: many distinct biological species demonstrate incomplete sorting of ancestral haplotypes. All of these criticisms, as well as those directed by better geneticists were provided on reviews of the report by Ramey et al. These criticisms were in regard to limitations of skin samples for sequence analysis; the low number of base pairs and, subsequently, microsatellites employed; the overly stringent criteria; and the incorrect application of various statistical tests. Ramey et al., who submitted the manuscript to *Animal Conservation* for publication without modification, ignored all of these important criticisms. I consider it a failure of the review process of *Animal Conservation* that such a flawed study was accepted for publication. Colleagues and I have detailed many of the failings of that paper in a short response to Ramey et al. (2005) submitted to *Animal Conservation* (Vigneiri et al., *in press*).

A major difference between King et al. and Ramey et al. (2005) is in sampling design. Ramey et al. (2005) used a large number of geographic samples, many from areas near or at subspecies boundaries, with a small number of samples per locality. King et al. instead used large numbers of individuals from fewer populations, generally away from subspecies boundaries (except perhaps the *campestris* and northern *pallidus* samples). I find that the design employed by King et al. is absolutely the more appropriate design, for the reasons given by King et al.

I'll admit that I interpreted the haplotypes reportedly "shared" by subspecies of *Z. preblei* (Ramey et al. 2005) as sorting errors. By sequencing a much larger region (1380 bp from 2 regions of mtDNA and 21 microsatellite DNA loci vs 346 bp of the mtDNA control region and 5 microsatellite loci), King et al. show that those "shared" haplotypes were simply the result of inadequate sequencing: in terms of too few

characters, and poor analytical techniques (e.g., poor quality assurance/quality control; attempts to confirm findings based on another region of mtDNA).

Ramey et al. (2005) represented, in my opinion, a perversion of the scientific method and the peer-review process. After its publication, I heard charges that the USFW was somehow guilty of collusion with Ramey et al., and that the paper's publication would be taken on face value by the USFW as proof that it was the "best available science." I'm proud of the USFW for seeking a resolution to the scientific debate by funding this comprehensive study by a federal laboratory. I suspect that Ramey, who describes himself as a "conservation genetics expert," will continue to declare that he is the only honest voice in the wilderness, and that all of his critics are politically motivated. In view of the fact that Ramey has been awarded by the current federal administration with a DOI contract as a "genetic consultant" and has announced his intention of targeting additional taxa for delisting, his motivation is quite clear. I may have been mistaken when I assumed that Ramey et al. (2005) merely represented shoddy science and stubbornness. The vast political and financial pressures that fuel the debate between an endangered subspecies of jumping mouse and developers should never influence the objective science of the issue.

I fully support the conclusions and interpretations presented in King et al.; I am extremely impressed with this study. I reviewed an earlier draft of King et al. This report includes the full information on *Z. p. luteus* (missing from the earlier draft), and is substantially improved in terms of revision of the tables and appendices. As I indicated on my review of the earlier draft, I would still clarify (as regards the sampling strategy) the need to sample away from subspecies boundaries when evaluation subspecific differentiation. Personally I doubt that a taxonomically more-comprehensive study of all subspecies of *Z. hudsonius* will indicate species status for the *luteus-pallidus* clade, a possibility suggested by King et al. However, I think there is much to be learned by a detailed phylogeographic analysis of the entire species, with an eye towards understanding the current disjunct distribution of the *luteus-pallidus* clade in contrast to the broad contact between *pallidus* and *intermedius*.

#### Specific Questions Posed in USFW Letter (26 January 2006):

1. *Were the appropriate methodologies and markers used?*—Yes.
2. *Do you support the authors' conclusions about the taxonomic validity of *Z. h. preblei* and neighboring subspecies?*—Yes.
3. *Do you support the authors' conclusions that *Z. h. preblei* is comprised of at least two distinct population segments worthy of individual management consideration?*—King et al. have demonstrated convincingly that there are indeed two genetically distinct groups within *Z. h. preblei*. In order to conserve the genetic diversity with the subspecies, both should receive management consideration. From what I recall of the excellent Recovery Plan drafted by the USFWS, it should not require any modification specific to each of these genetic groups, but should include both groups.
4. *Are there possible alternative interpretations of the data that could be drawn from the genetics data?*—None that I can envision.
5. *What additional analysis, if any, is needed to verify the study's assertions and why?*—The study is complete and exhaustive as it is.
6. *What are the most likely explanations for the conflicts between the conclusions of Ramey et al. (2005) and King et al. (2006)?*—I agree with the explanations provided by King et al. Ramey et al. used a poor sampling strategy, unrealistic criteria, inadequate sequence length, and an inadequate number of microsatellite loci. In addition to the lack of quality assurance/quality control efforts pointed out by King et al., I would add that Ramey et al.'s study most likely suffered from cross-contamination of laboratory samples (not uncommon in the sequencing of "rare" DNA). Basically, in my considered opinion, Ramey et al. (2005) is sloppy science. If the peer reviews received by *Animal Conservation* were anything like

the reviews that Ramey et al.'s report to the USFWS received, it should never have been published. In effect, *Animal Conservation* failed to protect Ramey et al. from themselves. This is a good reminder to the USFWS that just because something is published does not mean that it is necessarily the "best available science."